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# IMMUNOLOGICAL STUDIES ON SERA FROM PATIENTS WITH MALIGNANT TUMOR

by

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## I. INTRODUCTION

Since OUTERLONY[1] developed a double-diffusion method in a agar plate and then Grabar and WILLIAMS[2] introduced the method of immunoelectrophoresis, a lot of more detailed studies on the plasma proteins in neoplastic disease have been carried out by those methods. In this fields, Grabar and his co-workers[3] made, for the first time, an application of the immunoelectrophoresis for the investigation of human myelomatous proteins. Using the gel-diffusion technique, DARCY[4] has reported highly increased $\alpha$-globulin in the serum of rats bearing tumor and concluded that it was associated with rapidly growing tissue. CAMPBELL[5] et al. also reached to the similar conclusion in rats. J. Clausen et al.[6] studied on sera from mice carrying two transplantable plasma-cell leukemias and recognized the presence of $\gamma$-paraprotein in $\beta$-region. MIYAKE[7] has reported that the two paraproteins in sera from patients with leukemia would be a useful aid in differential diagnosis of three types of this disease. MILLER and BERNFELD[8] have studied an anomalous protein in plasma of C3H mice bearing spontaneous mammary adenocarcinoma which has the electrophoretic mobility of $\alpha$-globulin. Recently, by means of OUCHTERLONY's or GRABAR's technique, ISHIKAWA and TAKAYANAGI[9][10] have investigated abnormal proteins in sera from patients with gastric cancer or other malignant tumor and considered a possibility of immunochemical diagnosis of cancer.

However, there is a diversity of opinion as to the existence of specific component in serum from patient with malignant tumor.

The purpose of this paper is to study the variation of serum proteins in malignant tumors by immunoelectrophoretic analysis with absorbed antisera and to discuss the clinical availability of this method.

## II. MATERIALS AND METHODS

1) Materials. (a) Normal human serum (NHS) was obtained by pooling of blood from healthy donors and pilot tubes attached to Blood-Transfusion-Bial for cross-match. (b) Sera from patients: they were obtained generally from fasting patients before the surgical operation. Separation of serum was done after preserving them in 37°C for 30 minutes and then in a refrigerator overnight. (c) Ascitic fluids were collected from patients at the time of operation, and centrifuged to obtain the supernatants. Some of them were lyophilized for storage.

Total protein concentration of the materials was determined by Hitachi-Refractometer.

2) Immunoelectrophoresis (IEP). IEP was performed according to somewhat modified technique of SCHEIDEGGER's micro-method[11]. Rectangular glass plate (5.2×7.6 cm)[12] was cleaned thoroughly and coated[13] with 2 cc of 0.6% aqueous solution of Difico-Purified-Agar. Then, 5 cc of 1.2% agar in veronal buffer (pH8.2, $\mu=0.05$) was poured over this glass plate after it had been dried and laid in horizontal plane. Four holes (3 mm in diameter) were made in the gel which served as reservoirs for the materials to be analyzed (Fig. 1). Buffer system was divided into two vessels, i. e.

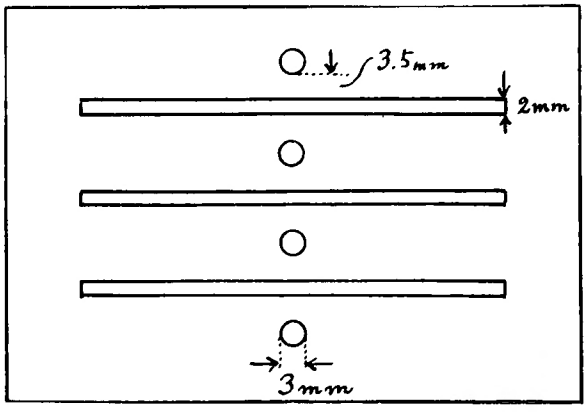



**Fig. 1** Placement of reservoirs in agar plate for modified Scheidegger's technique

electrode and buffer vessels; and U-tubes filled with 3% agar in buffer were used for their connection. Electrical contact of the agar plate with the buffer vessels was established by filter papers which were attached simply on both edges of the plates. Electrophoresis was carried out in a plastic box for 2～2.5 hours under constant current (1.6 mA/cm) and at a room temperature. After electrophoresis had been completed, three long slits were made in parallel with migration axis, and rabbit antiserum was poured into the reservoirs thus formed. The agar plate was then preserved in a moist chamber at a room temperature, and development of precipitation pattern was observed usally for 24 hours. For the staining of the precipitate lines, the agar plate was washed in physiological saline solution containing 0.01% merthiolate, for at least 4 days, and dried with cover of filter paper.

3) OUCHTERLONY's double-diffusion method. 1 vol. of veronal buffer (pH 8.2, $\mu=1.0$) was added to 3 vol. of physiological saline solution in which agar was dissolved with the proportion of 1.2%, and then an agar plate of 1 mm thick was prepared on the same glass plate as in IEP. Fig. 2 shows the form and dimension of reservoirs. These are the micromodification of OUCHTERLONY's method which was introduced by TACHIBANA.[14] When double-diffusion was performed in the modified method, development of precipitation pattern was completed usually within 24 hours. Washing

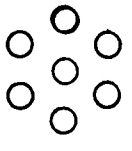


**Fig. 2** Micro-technique of Ouchterlony's method
Reservoirs: 3 mm in diameter, the distance between reservoirs: 3.5 mm

Fig. 3～8 IEP developed with anti-cancer-plasma rabbit serum before and after the secondary stimulus Non-absrobed antiserum in reservoirs at the left of the Fig., Absorbed one at the right. Antigen reservoirs; normal human serum (NHS) and cancer plasma (C-P).

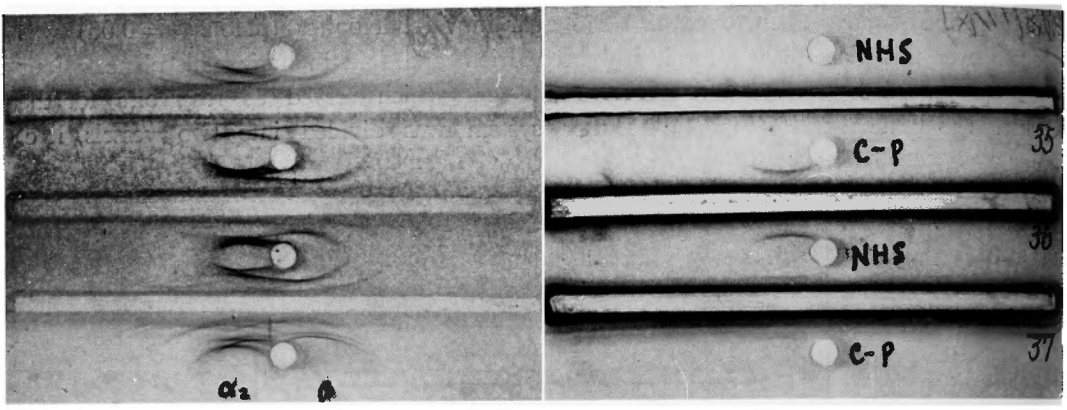



**Fig. 3** Before secondary stimulus (6w after primary stimulus)

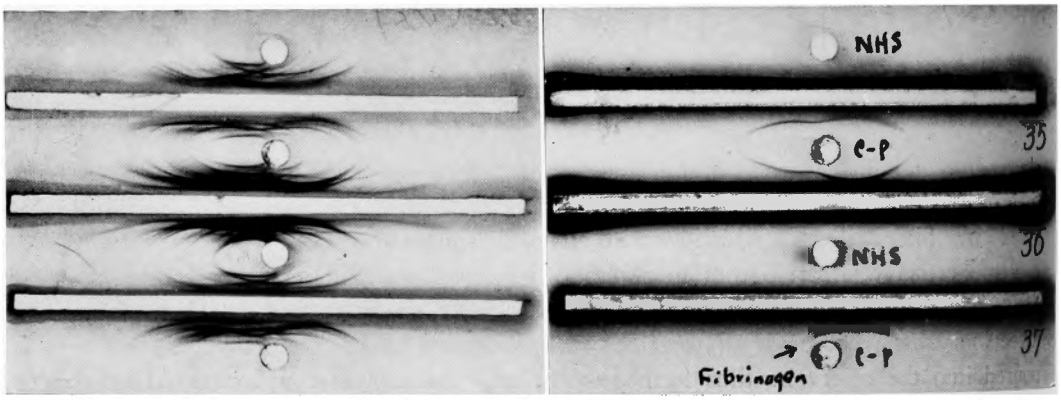



**Fig. 4** 4th day after the first injection (8w after primary stimulus).

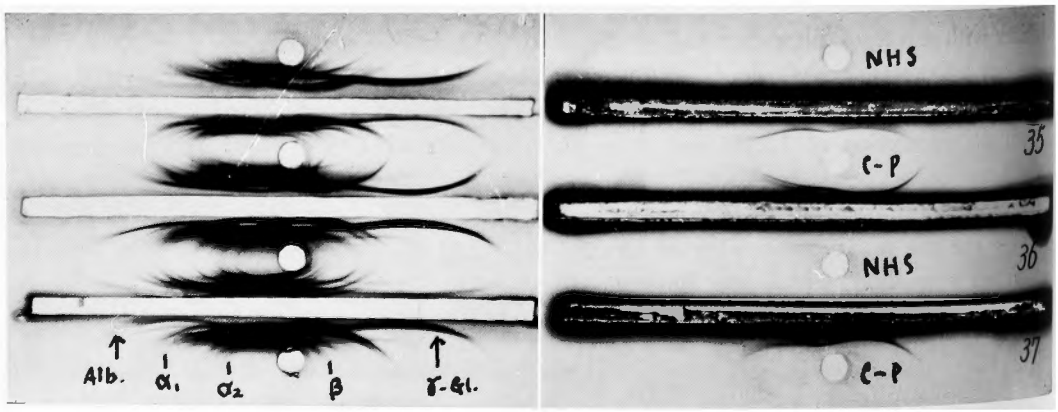



**Fig. 5** 3rd day after the second injection.

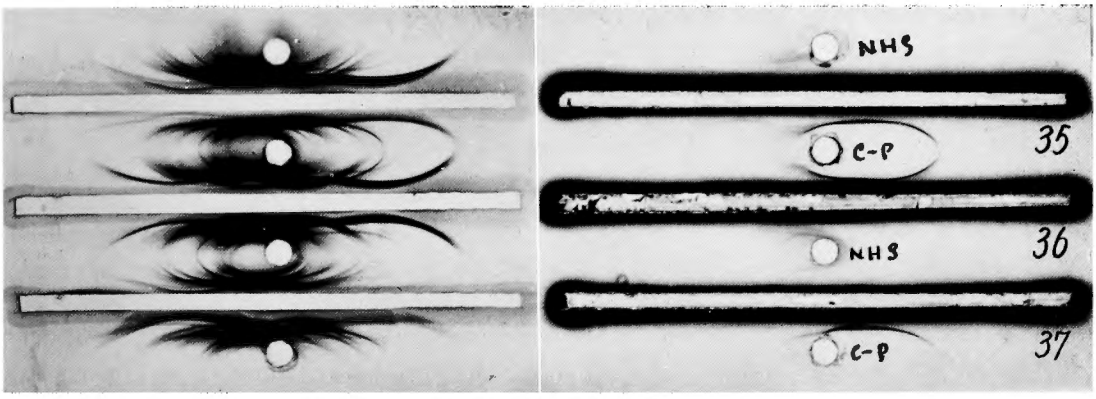



**Fig. 6** 5th day after the second injection. Marked increase in numbers of precipitate line is noticeable.

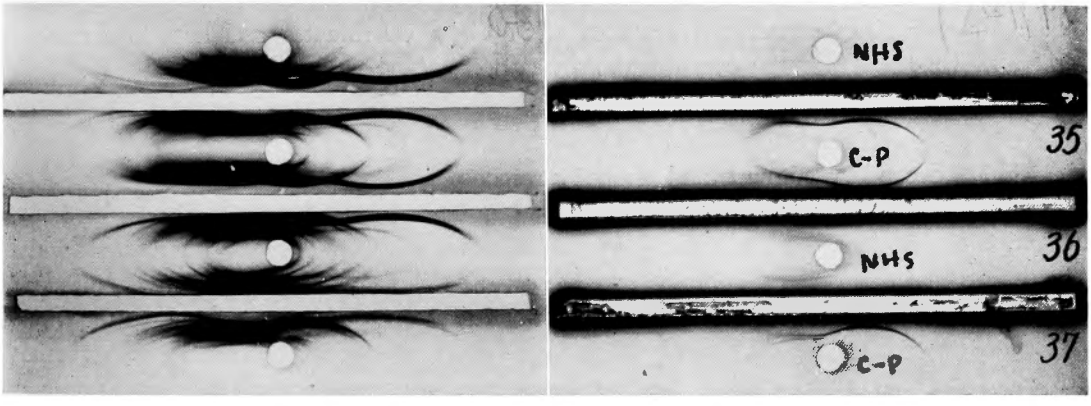



**Fig. 7** 7th day after the second injection. Continuance of prominent precipitation patterns except in γ-region developed with antiserum No. 37.

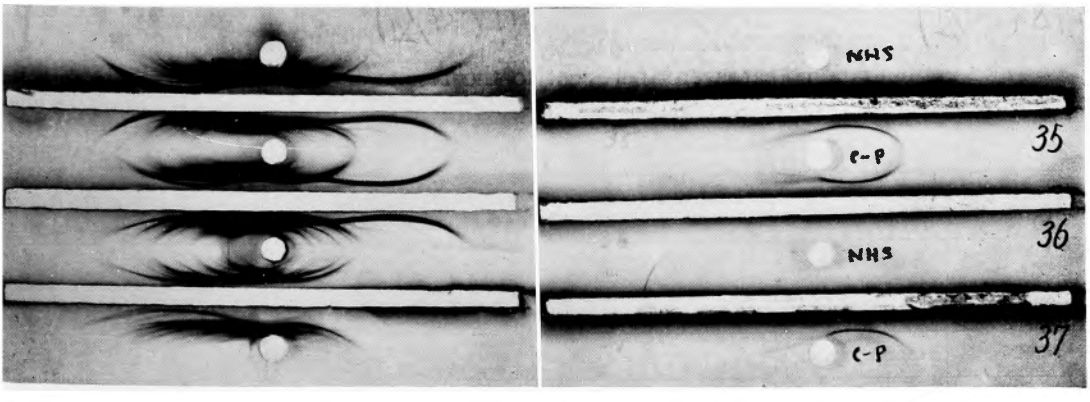



**Fig. 8** 4th day after the second injection in repeated "secondary stimulus" (13w after primary stimulus). Precipitation patterns in this Fig. are almost similar to those in Fig. 6 and 7.

and drying of the agar plate was carried out as described before.

4) Staining. Precipitate lines were stained usually by Amidoschwarz 10 B. For the staining of lipoprotein bow, saturated Sudan-Black B in 60% ethanol was available. In order to characterize glycoprotein-components, a staining technique was carried out after GRABAR's method.[13]

5) Antisera and their absorption by NHS. Rabbits (♂) were immunized with ascitic fluids, serum or plasma of cancer patients by intramuscular injection. The materials injected were emulsified with the equal parts of FREUND's[15] adjuvant that was consisted of liquid paraffin 8.5 vol., Arlacel A 1.5 vol. and kill-dried tubercle bacilli 2 mg/cc of liq. paraffin. As a primary stimulus 100 mg of total protein was injected twice with an interval of one week. Two or three months later, secondary stimulus was given by subcutaneous injection and rabbits were bled to death. Antiserum was separated after incubating at 37°C for 30 minutes and then keeping in a refrigerator overnight, and stored adding merthiolate to 0.01%.

Absorption of antisera. Various amounts of NHS from 0.05 cc to 0.6 cc were added to 1 cc of an antiserum, incubated at 37°C for 30 minutes and kept in a refrigerator overnight. After that centrifugation was done.

## III. RESULTS

§ 1. Secondary stimulus and antibody response

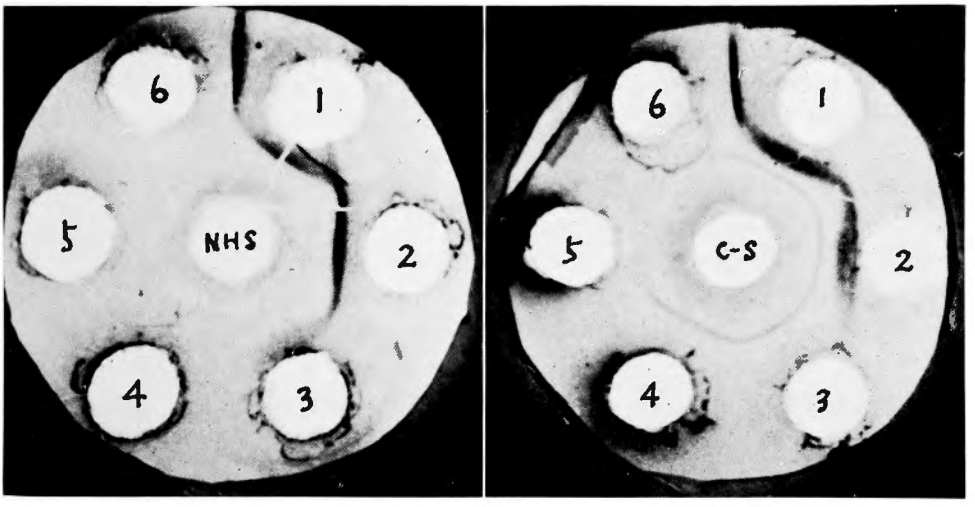
Progress of antibody response after secondary stimulus is shown in Fig. 3, 4, 5, 6, 7 and 8. The antisera in these cases were obtained from rabbits immunized with cancer plasma. Secondary stimulus was given as follows. Each rabbit was injected subcutaneously with cancer plasma (total protein 50 mg). Four days after the first injection the same procedure was followed (second injection). Antisera from partial bleeding were subjected to IEP. Absorption in this experiment was carried out according to the method of DRAY and YOUNG.[16] As the results, (1) time lag in the phase of antibody response was appeared between each antibody, (2) differences between individual rabbits were also observed. In case of the immunization of rabbits against human plasma, however, it may be expected that the most suitable day for total bleeding falls between 4th～7th day after the second injection.

The immunization that were pertinent to the results and discussions are described in Table 1. In all cases secondary stimulus was given by the same method.

**Table 1** Immunizations

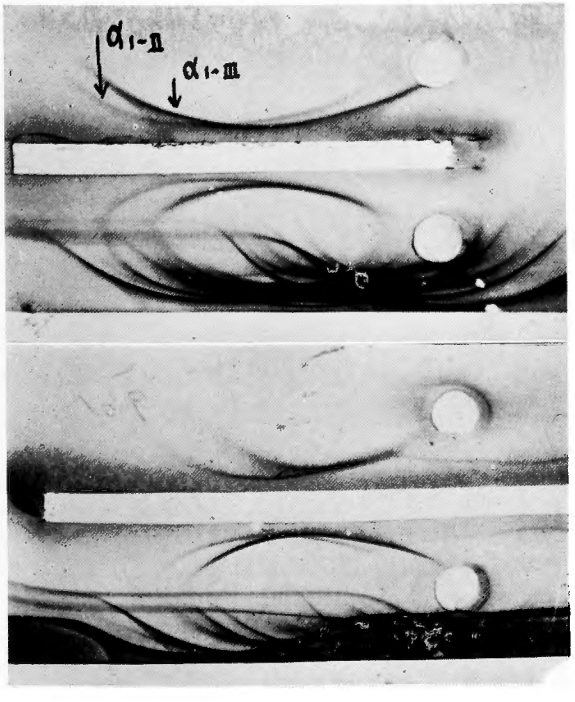
| No. | Material injected | Numbers of rabbits immunized | Secondary stimulus after following interval | Day of bleeding after 2nd injection |
|---|---|---|---|---|
| 7 | cancer ascites | 3 | 12w | 4th |
| 8 | cancer serum | 3 | 8w | 4th |
| 26 | cancer ascites | 6 | 9w | 5th |
| 35<br>36<br>37<br>pooled | cancer plasma | 3 | 10w | 5th |

**Fig. 9** Analysis of antiserum No. 7 absorbed with NHS at different absorption ratio (enlarged figures of micro-technique of Ouchterlony's method)




Antigen : center left (NHS) ; center right (cancer serum)
Antiserum : absorbed No. 7 in periphery
Absorption ratio :- (1) 1 : 0.05, (2) 1 : 0.1, (3) 1 : 0.2, (4) 1 : 0.3, (5) 1 : 0.4, (6) 1 : 0.6.

**Fig. 10** IEP of sera from patients with neoplasmas developed with absorbed antiserum No. 7




○ gastric cancer

←— absorbed No. 7

○ NHS

←— non-absorbed No. 7

○ NHS

←— absorbed No. 7

○ fibrosarcoma

←— non-absorbed pooled antiserum

§ 2. Clinical observations by IEP with absorbed antisera

1) Absorption and IEP with antiserum No 7

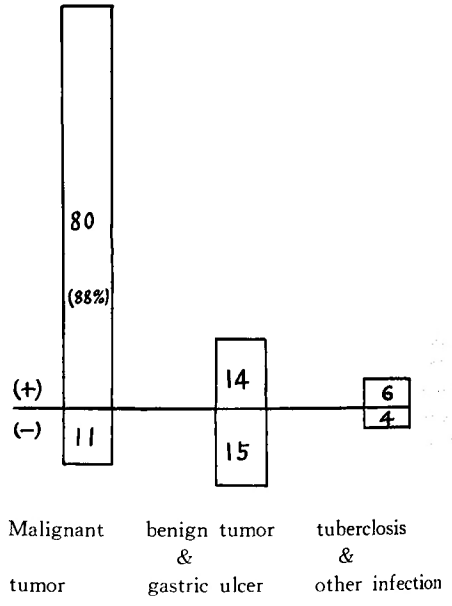
After antiserum No 7 was absorbed with NHS, OUCHTERLONY's method was performed between the absorbed antiserum No. 7 and cancer serum, simultaneously accompanied by control reaction between the absorbed antiserum No. 7 and NHS Fig 9 shows the reaction pattern An extra line was found between absorbed antiserum No 7 and cancer serum which was absent at control. Absorption ratio that gave only an extra line was decided to be 1 : 0 15 (antiserum : NHS).

Immunoelectrophoretic tracings shown in Fig. 10 were developed with absorbed antiserum No. 7. The single precipitation line on OUCHTERLONY's methods was divided into two lines on IEP. They were also present at control serum (NHS), although somewhat fainter than at cancer serum The two lines thus obtained at cancer serum were termed as $\alpha_{1-I}$ and $\alpha_{1-II}$ respectively. In cancer serum the bow of $\alpha_{1-II}$ became clearer and larger, and crossed over $\alpha_{1-I}$ bow (Fig. 10). Perhaps a component shown by $\alpha_{1-II}$ bow was increased in cancer serum than in NHS, meanwhile a component of $\alpha_{1-I}$ was relatively stationary. The clinical cases of prominent $\alpha_{1-II}$ were summarized in Table 2 and Diagram 1. As shown in Diagram 1, positive cases amounted to 88% in malignant tumor, but they were also high in groups of other diseases. Therefore it was not possible to discriminate cancer from other diseases by absorbed antiserum No. 7. Although the extent of this work was not enough to discuss the correlation between the immunological data and histological types or their malignancy, the prevalence of prominent $\alpha_{1-II}$ bow was observed in cases of liver metastasis of gastric cancer. Negative results were obtained in a very early stadium of gastric cancer as well as a few cases of end stadium.

**Table 2** Classification of materials from patients with malignant tumor

| Site of tumor | Total | Result of IEP (+) | Result of IEP (−) |
|---|---|---|---|
| Stomach | 46 | 40 | 6 |
| Colon & Rectum | 8 | 7 | 1 |
| Breast | 7 | 6 | 1 |
| Lung | 4 | 4 | 0 |
| Esophagus | 4 | 4 | 0 |
| Pancreas | 3 | 3 | 0 |
| | 5 | 4 | 1 |
| Miscellaneous | 14 | 12 | 2 |
| Result of IEP with absorbed No. 7 | | 80 (88%) | 11 (12%) |

**Diagram 1** Comparison of the results from IEP of pathological sera, developed with absorbed antiserum No. 7




2) IEP developed with absorbed antiserum No. 8

Antiserum No. 8 absorbed with NHS (1 : 0.15) has showed two precipitate lines in

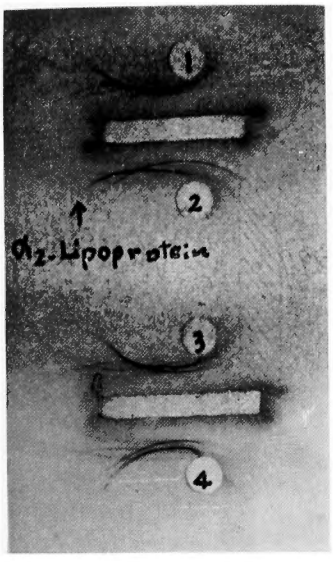



**Fig. 11** IEP of pathologic sera developed with absorbed antiserum No. 8

(1) Gastric cancer

(2) Gastric cancer

(3) Gastric ulcer

(4) Mediastinal tumor

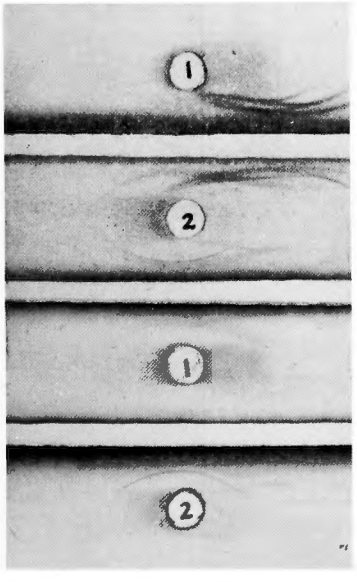



**Fig. 12** Analysis of antiserum No. 26 absorbed with NHS at different absorption ratio

Absorption ratio :- (26-a) 1 : 0.1,
(26-b) 1 : 0.2,
(26-c) 1 : 0.3.

26-a

Antigen : (1). NHS
(2). cancer serum

26-b

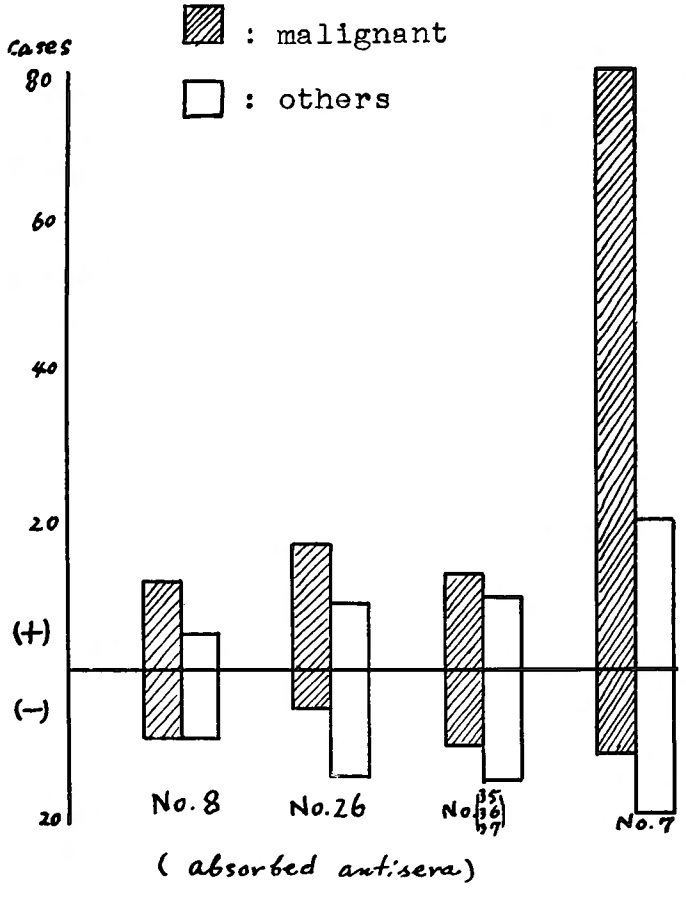
26-c

$\alpha_2$-region. One of them was clearly stained by Sudan-Black B and the other unstainable. Henceforth the former was designated as $\alpha_2$-lipoprotein. The latter appeared frequently as a large bow in cancer sera than in NHS (Fig. 11), and sometimes was accompanied by $\alpha_2$-lipoprotein line But in 4 cases of cancer sera the SBB-unstainable $\alpha_2$-line was observed independently. The data of clinical observation by absorbed antiserum No. 8 is present in Diagram 2.

3) IEP developed with absorbed antiserum No. 26

Figure 12 shows the precipitation pattern developed with absorbed antiserum No. 26. When absorption ratio was 1 : 0.3, a fine precipitate line appeared in $\alpha_2 \sim \beta$-region in

**Diagram 2** Comparison of the results from IEP developed with absorbed antisera



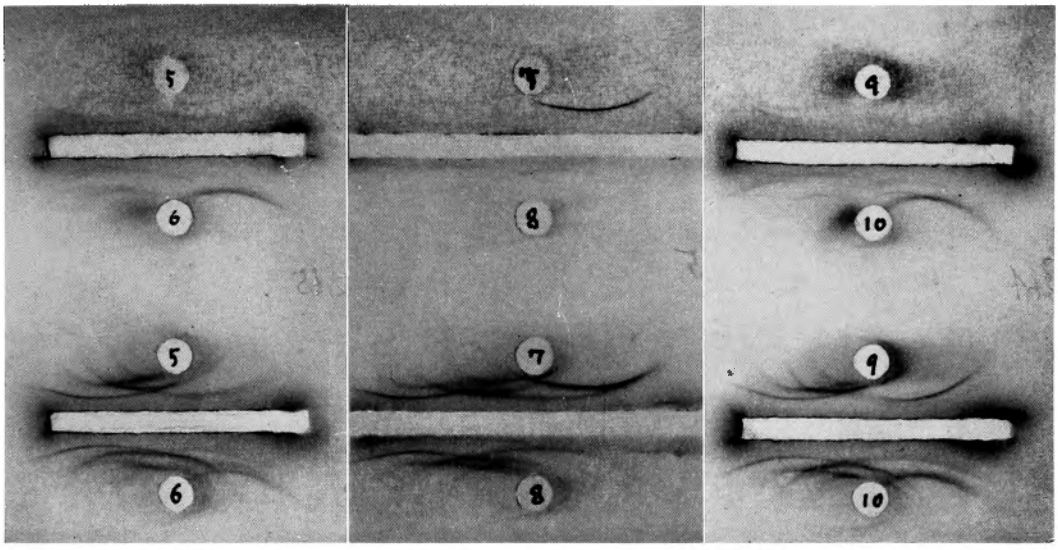

serum from a patient with lung cancer, while it was absent in NHS. However, appearance of this line in cancer sera is so undefinite that the differential diagnosis of cancer may be not possible.

Precipitation patterns developed with absorbed antiserum No. 26 and anti-cancer-plasma rabbit serum are showed in Fig. 13. Data from clinical observation is shown in Diagram 2.

4) IEP developed with absorbed antiserum which was pooled from the rabbits immunized against cancer plasma

In order to observe minor components in $\alpha$- and $\beta$-regions absorption ratio was settled at 1 : 0.2. Frequently there was a bold line of precipitate in $\beta$-region, and it often appeared in cancer serum (Fig. 13). In addition to this line, a fainter and smaller bow in $\beta$-region sometimes appeared concurrently with a similar bow that was developed with abs. No. 26. So it was presumed that these antisera (No. 26 and pooled anti-caneer-plasm aserum) contained more than two antibodies against extra component. One of them was an uncommon antibody and the other was a common one. From the observationon on the various pathologic sera, the precipitate lines of this group were not specific for cancer serum but seemed to be a group-specific component (Hirschfeld) or the

**Fig. 13** IEP of pathologic sera developed with absorbed antiserum No. 26 and absorbed anti-cancer-plasma rabbit seum




Antiserum: upper reservoir-abs. No. 26
lower reservoir-abs anti-cancer-plasma

Antigen: (5) gastric cancer (7) gastric ulcer (9) stuma nodosa
(6) gastric cancer (8) breast cancer (10) rectal cancer

representatives of some other conditions than cancer. Result of IEP with this absorbed antiserum is shown in Diagram 2.

§ 3. Characterization of the precipitate line developed with absorbed antiserum No 7

The relation between $\alpha_{1-\text{III}}$ and ammonium sulfate fractions of cancer ascites was studied. The supernatant and the sediment of ascites fractionated by half-saturated ammonium sulfate were dialyzed by cold tap water and then by physiological saline Each protein concentration was adjusted to 4% by 20% polyethyleneglycol solution in physiological saline. Immunoelectrophoretic tracings in Fig 14 demonstrate a reaction between the fractions above mentioned and absorbed antiserum No 7. The bow of $\alpha_{1-\text{III}}$ that had been already observed in cancer serum was also clearly developed on the supernatant, while very obscure one was developed on the sediment. It is assumed, therefore, that $\alpha_{1-\text{III}}$ bow is a representative of acid mucoproteins These results led to further experiment to elucidate the relation between $\alpha_{1-\text{III}}$ and acid mucoproteins ($M_1$ and $M_2$) separated dy paper electrophoresis at pH 4 4.

As the method of paper electrophoresis, the technigue was employed which has been described by YAMAGUCHI[17]. Briefly, 0.05 cc of serum was applied to the center of each strip (2 cm in width) of Toyo Roshi No. 51 filter paper, and electrophoresis was carried out in the phosphate buffer (pH 4.4, $\mu=0.2$), at constant voltage (3 vol/cm) for 12 hours and at a room temperature. Immediately after electrophoresis two strips were removed from the cell. One of them was stained with Amidoschwarz 10 B and the other with PAS staining. Figure 16 shows the stained strips. The remaining five strips were cut into three pieces corresponding to M-1, M-2 and A segments of stained strips. Each piece of

**Fig. 14** IEP of the fractions from cancer ascites treated with 1/2-saturated ammonium sulfate $\alpha_{1-III}$ is demonstrable on the supernatant, but scarecely developed on the sediment.

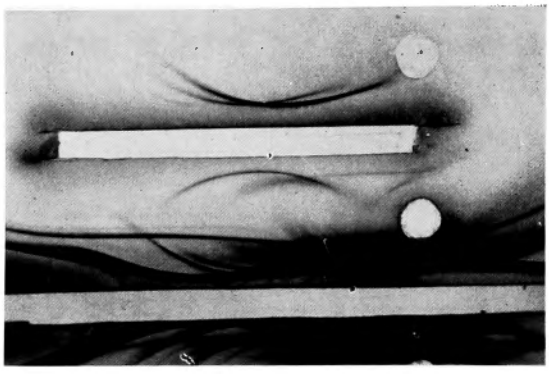



**Fig. 15** Demonstration of common antigenicity between αcomponent and M-2 fraction

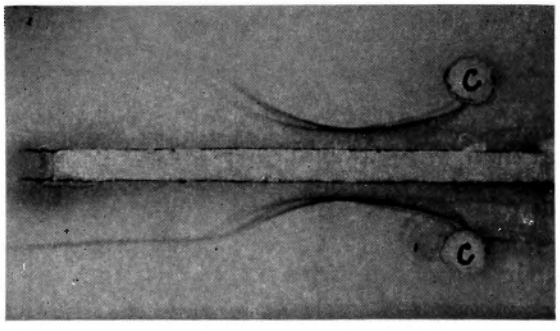
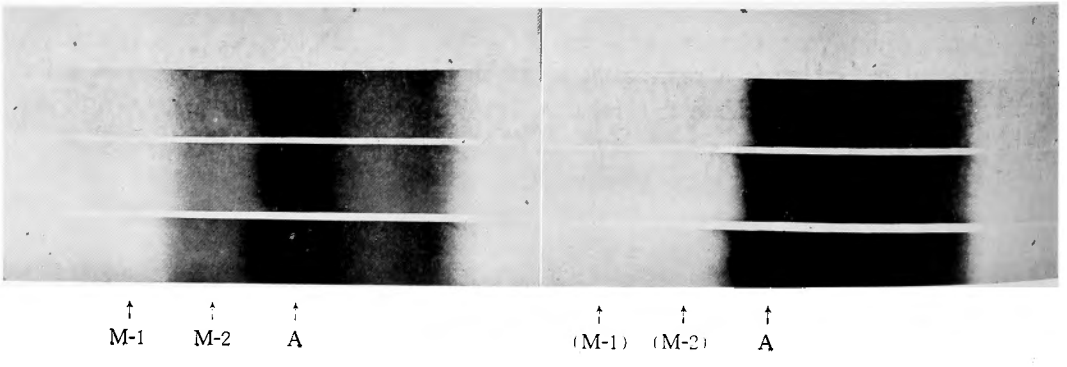



**Fig. 16** Paper electrophoregram of cancer serum and NHS (pH 4.4, $\mu = 0.2$)
Staining upper one by PAS reaction lower three by Amidoschwarz 10 B



same segment was put together into one jar, and 5 cc of physiological saline was added. Both jars containing M-1 and M-2 respectively were placed in a refrigerator for 12 hours, and then pieces of filter paper were discarded. After repeating the same procedure for 10 times, M-1 and M-2 were collected as mentioned above and dialyzed by physiological saline. Subsequently they were condenced by 20% polyethyleneglycol saline solution up to the original volume before electrophoresis. In order to elucidate the relation between $\alpha_{1-\text{II}}$ and M-1 or M-2, the method described by OSSERMAN[18] was adopted. Namely, after electrophoresis of cancer serum had been completed in the agar-plate, preparations of M-1 and M-2 were poured into outer reservoirs respectively, while the absorbed antiserum No. 7 was poured into middle reservoir. Accompanied by development of the bows of $\alpha_{1-\text{II}}$ and $\alpha_{1-\text{III}}$, a single straight line of precipitate was found between antiserum reservoir and M-2 reservoir and moreover confluence of the line with $\alpha_{1-\text{III}}$ bow was observed. On the other hand, such a line was not found at M-1 reservoir

By PAS reaction some precipitate lines were slightly stained and both $\alpha_{1-\text{II}}$ and $\alpha_{1-\text{III}}$ belonged to the stained lines.

## IV. DISCUSSION

1) As to the method of IEP

In this study Scheidegger's micro-method was slightly modified. For the convenience of manipulation as well as of combination of different antisera, glass plate (5.2×7.6 cm) was employed (MATSUHASHI). The dimensions of both antigen and antibody reservoirs and the distance between each reservoir are important in connection with precipitation pattern. The dimensions (Fig. 1) applied in the experiment with the absorbed antiserum No. 7 has produced a good result. These conditions were also proved to be available in other absorbed antisera. Large antigen reservoir seemed to be suitable to analyze the minor component in human serum with absorbed antiserum. As to dimensions of reservoirs, author's result was identical with description of TAKAYANAGI[19]. Antigen excess pattern was not concerned in minor component but albumin. As a matter of course, antigen reservoirs should be placed in a single line rectangular for migration axis. Even if absorbed antiserum was used, two reservoirs that were placed in accordance with migration axis would not bring satisfactory result, owing to the change of precipitation patterns. When electrophoresis was carried out, constant current was used to produce a stable condition.

2) Immunization and antibody response

From the study of the antibody production in the rabbits injected with egg-albumin, Y. MIYAKE[20] stated that Freund's adjuvant method, especially intramuscular double injections of adjuvant mixture were proved to be the best method of immunization. According to this report rabbits were immunized with ascites, serum or plasma proteins of cancer patients, and antisera were tested by IEP. Contrary to expectation, only scanty precipitate lines were found in $\alpha_2$ and $\beta$-regions at 6 or 8 weeks after the immunization. Accordingly, secondary stimulus was given by subcutaneous injection to obtain high titer antiserum. Immunization procedure in secondary stimulus was those of described before. From the observation on progress of antibody response, the marked increase of precipitate

lines was noticed in antisera obtained on the 4th～7th day after the second injection. In immunization wtih "Mucoprotein Fraction"[21] (Mp) almost same patterns of secondary response was obtained. That is to say, 5 months after the primary stimulus 1 mg of Mp was injected to 6 rabbits according to the procedure described above. The antiserum titer was measured by the dilution technique in the Ouchterlony's method. Two of six antisera gave the highest titer on 5th day after the second injection, while the other four unehanged. About the interval of injection in secondary stimulus and the maximum serum antibody concentration, DIXONet al.[22] reported that the serum antibody concentration attained to the maximun on 5th～7th day after the injection considering the results of the experiment on anamnestic antibcdy response of rabbit against serum protein antigen. N. TANAKA[23] reported that antibody titer in secondary response was far higher than in primary response in the immunization against diphtheria toxoid and that the mode of secondary response depended upon the individual difference of animals treated, method of injection and nature of antigens. On the other hand, studying on the precipitin production in rabbits injected with an azoprotein, HEIDELBERGER et al.[24] concluded that the day of the maximum antibody production was altered from the 3rd to 5th day according to sensitizing quantity in the last injection. In general, it is also accepted that type specific polysaccharide of Pneumococcus does not react against secondary stimulus. On the basis of these experiments and the reports, it may be concluded that antiserum after secondary stimulus si rich in antibody and is available for immunoelectrophoretic analysis of human serum. But as to some specific antigen-aatibody systems further experiment is necessary.

In regard to the relation between specificity of antiserum and stage of immunization, it is generally accepted that the specificity of antiserum is prominent in early stage than in late stage. K. SUZUKI and Y. MORI,[25] however, have reported the adverse phenomenon in rabbit antiserum immunized with horse serum. It still remains to be analyzed how the problem is concerned with antibody production due to secondary response as it was observed in this experiment.

3) Mucoprotein

As to plasma mucoprotein levels in cancer patients, Winzler and Smith[26] had already reported that the materials responcible for the increased mucoprotein levels in pneumonia and cancer (and probably other condition) were chemically similar to the mucoprotein that were isolated from normal human plasma. K. TSURUMI[27] stated that the possibility of an increase in specific sialic acid-rich mucoprotein fraction in plasma or urine of cancer patients might not be negligible. Recently YAMAGUCHI et al.[28] have reported the polarographic characteristic of M-1 fraction that was separated by paper electrophoresis at pH 4.4, and stated that M-1-P. P. test (M-1-paper electrophoresis-polarography test) was made with better result than Brdicka's filtrate test in the detection of cancer.

In the present studies the prevalence of prominent $\alpha_{1-\text{III}}$ bow was observed in sera from patients with malignant tumor and in lung tuberclosis as well as other pathologic conditions. Such a cross-phenomenon of $\alpha_1$-mucoprotein was already reported by TAKAYANAGI and MIYAKE. Consequently, it was interesting to investigate the relation between $\alpha_{1-\text{III}}$ and acid mucoproteins i. e. M-1 and M-2. As described before, the data showed that $\alpha_{1-\text{III}}$ had a common antigenicity with M-2 fraction, and that it was soluble in half-

saturated ammonium-sulfate. Antibody against M-1 was not demonstrable in the present experiment.

Concerning to the character of M-2 Mehl, et al.[29] observed a concomitant increase in M-2 with M-1 in sera from patients and they described it as a kind of mucoproteins. On the other hand Markham[30] described that M-1 and M-2 travelled with $\alpha_1$ and $\alpha_2$-globulins respectively on electrophoresis at pH 8.6. About to the electrophoretic mobility of M-2, Schmidt[31] has also described it as $\alpha_2$-glycoproteins. So the nomenclature of $\alpha_1$-Ⅲ (immunologically common to M-2 fraction) in this study should be discussed here. In the present experiment, IEP was performed at pH 8.2. The precipitation patterns that were developed with both absorbed antiserum No 7 and pooled antiserum were compared to single electrophoresis, and two precipitate lines thus obtained were tentatively designated as $\alpha_1$-Ⅰ and $\alpha_1$-Ⅲ respectively. According to the nomenclature of HIRSCHFELD [32] four precipitate lines were attributed to $\alpha_1$-globulin. Comparing the position of lines, it was presumed that $\alpha_1$-Ⅲ was corresponding to $\alpha_1$-glycoprotein in his description. In short, it will be necessary to investigate whether main component of M-2 fraction should migrate with $\alpha_1$-globulins at pH 8.2, and further, whether $\alpha_1$-Ⅲ might be a subfraction of M-2 which indicated the electrophoretic mobility of $\alpha_2$ globulins at pH 8.6.

Recently, M. L. Petermann[33] has reviewed the disturbances that may occur in the plasma proteins in neoplastic diseases. In this review we found the disappointing results as follows:

(1) abnormal findings in patients with advanced cancer,
(2) abnormal findings in patients with other wasting disease or with infection,
(3) negative findings in patients with early cancer with rare exeptions.

These descriptions are almost entirely applied to the results obtained with absorbed antiserum No. 7. B. ROBERT, et al.[34] have indicated that polarographic analysis was superior to immunoelectrophoretic analysis for the investigation of changes in molecular moiety of mucoprotein. From all these data there may be very little hope in detecting an "specific" mucoprotein in cancer serum by means of immunoelectrophoretic analysis.
Concerning to $\alpha_2$ and $\beta$-regions

In the present studies remarkable precipitate line was observed neither in $\alpha_2$ nor in $\beta$-region. Some components that were developed in those regions with absorbed antisera were not characteristic for malignant tumor. It still remains to elucidate the relation between the observed patterns and C-reactive protein.

As increase in fibrinogen level in cancer plasma has been reported[35][36], especially in early stage on malignancy[37], the possibility of detection of fibrinogen or its degradation products in serum offers a further interest in a gel-diffusion method.

## V. SUMMARY

Sera from patients with malignant tumor were observed by IEP, and a mucoprotein component characteristic for malignant tumor was investigated.

Rabbits (♂) were immunized with ascites, serum or plasma from patients with malignant tumor. 2 or 3 months later, secondary stimulus was given and rabbits were bled to death. Antisera were absorbed with pooled normal human serum and subjected

to immunoelectrophoretic reaction with cancer serum and normal serum. Four absorbed antisera were selected that were supposed to contain some antibodies against cancer-characteristic components. With those absorbed antisera materials from patients were tested by IEP. Summary of the results in present experiment are as follows.

(1) Although primary stimulus was given to rabbits with Freund's adjuvant. secondary stimulus was required to obtain high titer antiserum.

(2) In case of double subcutaneous injection as secondary stimulus it may be concluded that the day of the maximum antibody content falls between 4th～7th day after the second injection.

(3) Absorbed antiserum No. 7 developed two precipitate lines in $\alpha_1$-region both to cancer and normal serum, and they were designated as $\alpha_1$-Ⅰ and $\alpha_1$-Ⅱ respectively.

(4) The component of $\alpha_1$-Ⅱ showed the common antigenicity with M-2 fraction separated by paper electrophoresis at pH 4.4, and it was soluble to half-saturated ammonium sulfate. Accordingly it may belong to acid mucoproteins.

(5) The prevalence of prominent $\alpha_1$-Ⅱ bow was observed in 80 cases of 91 patients with malignant tumor.

(6) Differential diagnosis of cancer from other diseases was impossible by findings of $\alpha_1$-Ⅱ bow.

(7) In $\alpha_2$ and $\beta$-regions no characteristic component was observed by the present antisera.

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和文抄録

# 悪性腫瘍患者血清の免疫学的研究

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Grabar 及び Williams が，複雑抗原の解析に免疫電気泳動法を応用して以来，悪性腫瘍患者体液の抗原分析も詳細にすすめられるようになつた．そして最近，この方法により，悪性腫瘍患者の体液中に特異的な抗原因子を指摘出来るとの報告が出現している．

著者はその点を吟味するために，癌患者から得た腹水，血清または血漿で家兎を感作し，その抗血清を正常人血清で吸収した後に，これを免疫電気泳動法に応用して，癌患者血清に対して特徴的な沈降線を示す抗体を含むと考えられた吸収抗血清4種を撰択し，これらの吸収抗血清と患者血清との反応を免疫電気泳動法で追求したが，その結果下記の事項を認識した．

① Freund's adjuvant を併用し，2回筋注法で家兎を感作したが，全採血には更に，二次感作を必要とした．

② 二次感作に際して，総蛋白量50mgの血漿を皮下に4日間隔で2回注射すると，2回目の注射から4～7日の間が，全採血に適当な期間となる如くである．

③ 癌性腹水を以て感作して得た抗血清 No. 7 を正常人血清で吸収した後，Ouchterlony 法で検すると，癌血清にのみ現れる1本の沈降線を認めたが，免疫電気泳動法によれば，2本の線となり，正常人血清に対しても双方の線を認めた．そこでその各々を，$\alpha_1$-II，$\alpha_{1-III}$と名付けた．

④ この$\alpha_{1-III}$の因子はpH4.4の濾紙電気泳動法で分離されるM-2分画に対して，共通な抗原性を有するものと考えられたが，さらにまた硫安1/2飽和上清中に含まれることから，酸性糖蛋白の一種であろうと推定される．

⑤ この$\alpha_{1-III}$は悪性腫瘍患者血清91例中80例において，大きな弧状の沈降線となり$\alpha_{1-I}$の弧と交叉する所見を示した．しかし，炎症性疾患・良性腫瘍患者血清においても同様の変化を認めうるので，この沈降線を以て悪性腫瘍を鑑別診断することはできない．

⑥ 現在までの抗血清では，$\alpha_2$域及びβ域に関して患癌者血清に特徴的な沈降線は見出し得ないと云いたい．